

***In Silico* Toxicology: Simulating Interaction Thresholds for Human Exposure to Mixtures of Trichloroethylene, Tetrachloroethylene, and 1,1,1-Trichloroethane**

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In this study, we integrated our understanding of biochemistry, physiology, and metabolism of three commonly used organic solvents with computer simulation to present a new approach that we call “*in silico*” toxicology. Thus, we developed an interactive physiologically based pharmacokinetic (PBPK) model to predict the individual kinetics of trichloroethylene (TCE), perchloroethylene (PERC), and methylchloroform (MC) in humans exposed to differently constituted chemical mixtures of the three solvents. Model structure and parameterization originate from the literature. We calibrated the single-compound PBPK models using published data and described metabolic interactions within the chemical mixture using kinetic constants estimated in rats. The mixture model was used to explore the general pharmacokinetic profile of two common biomarkers of exposure, peak TCE blood levels and total amount of TCE metabolites generated, in rats and humans. Assuming that a 10% change in the biomarkers corresponds to a significant health effect, we calculated interaction thresholds for binary and ternary mixtures of TCE, PERC, and MC. Increases in the TCE blood levels led to higher availability of the parent compound for glutathione conjugation, a metabolic pathway associated with kidney toxicity/carcinogenicity. The simulated change in production rates of toxic conjugative metabolites exceeded 17% for a corresponding 10% increase in TCE blood concentration, indicating a nonlinear risk increase due to combined exposures to TCE. Evaluation of metabolic interactions and their thresholds illustrates a unique application of PBPK modeling in risk assessment of occupational exposures to chemical mixtures. **Key words:** chemical mixtures, computational toxicology, *in silico* toxicology, interaction thresholds, metabolic interaction, methylchloroform, occupational exposure, PBPK modeling, tetrachloroethylene, trichloroethylene. *Environ Health Perspect* 110:1031–1039 (2002). [Online 3 September 2002] <http://ehpnet1.niehs.nih.gov/docs/2002/110p1031-1039dobrev/abstract.html>

Trichloroethylene (TCE), tetrachloroethylene [perchloroethylene (PERC)], and 1,1,1-trichloroethane [methylchloroform (MC)] are volatile organic solvents of widespread industrial use. Their release into the environment occurs during manufacturing processes as well as with the normal use of these products. Combinations of the three chemicals have been frequently identified in contaminated groundwater in the proximity of production facilities and/or hazardous waste disposal sites (Mumtaz et al. 1998). Consequently, human occupational and/or environmental exposures to differently constituted mixtures of the solvents are likely to occur (Wu and Schaum 2000). TCE, PERC, and MC are high-priority chemicals reported by the Agency for Toxic Substances and Disease Registry under the Comprehensive Environmental Response, Compensation, and Liability Act with “Completed Exposure Pathway” (ATSDR 1997).

Coexposure to multiple chemicals may significantly affect the pharmacokinetics of one or more mixture components and alter the target tissue dose of the toxic moiety (Krishnan et al. 1994a, 1994b). The change in the tissue dosimetry caused by pharmacokinetic interactions depends on the relative concentrations of the mixture components

and the underlying mechanism of interaction. Competitive metabolic inhibition is the predominant mechanism of pharmacokinetic interaction for exposures to TCE in combination with other organic solvents (Andersen et al. 1987; Barton et al. 1995; Dobrev et al. 2001; El-Masri et al. 1996a, 1996b). Clearance by exhalation and metabolism are the two major routes of elimination for volatile organics from the body (Andersen 1981), and quantitative changes in any of those pathways are expected to significantly affect the chemicals’ pharmacokinetics. In humans, the metabolic clearance of PERC and MC is very low (IARC 1999; Monster 1979; Nolan et al. 1984; Reitz et al. 1988, 1996; Ward et al. 1988), and exhalation is the major route of elimination. Therefore, metabolic inhibition of MC and PERC caused by TCE coexposure will have only a minor impact on the overall kinetic behavior of these two compounds. TCE, on the other hand, is a highly cleared compound with toxicities clearly associated with its biotransformation (Gargas et al. 1990; Lash et al. 2000). Thus, the main focus of our study was to quantitatively assess the magnitude of pharmacokinetic changes in TCE pharmacokinetics due to the presence of the other two solvents in chemical mixtures.

The assessment of both acute and long-term occupational exposures to chemical mixtures containing TCE requires two different approaches. The major acute effect of TCE is on the central nervous system, and neurotoxic effects such as headaches, sleepiness, fatigue, and/or drowsiness occur at concentrations of approximately 100 ppm (Barton and Clewell 2000; Barton and Das 1996). Drowsiness may be caused by TCE itself and/or trichloroethanol (TCOH), a metabolite with a known sedative effect. Because both TCE and TCOH rapidly equilibrate with the highly perfused lipid-rich brain tissue, peak arterial blood TCE concentration (usually at the end of the exposure period) is a more appropriate dose metric for drowsiness and the sedative effects of TCE. The American Conference of Governmental Industrial Hygienists established a threshold limit value (TLV) of 50 ppm, intended to protect against fatigue, headache, and irritability (ACGIH 2000).

Long-term exposures to TCE have been repeatedly shown to induce several types of tumors in experimental animals (reviewed in Barton and Clewell 2000; Bull 2000; Lash et al. 2000), and all of them appear to be caused by metabolism-related products. TCE is metabolized by two metabolic pathways: cytochrome P450-mediated oxidation and glutathione-S-transferase (GST)-mediated conjugation with glutathione (GSH; Figure 1; Lash et al. 2000). The oxidative metabolism of TCE leads to formation of a variety of products, among which chloral hydrate (CHO), trichloroacetic acid, and dichloroacetic acid are considered carcinogenic in rodents. The

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This work was conducted at the Center for Environmental Toxicology and Technology at Colorado State University. Helpful discussions with J. Dennison are gratefully acknowledged.

The research was supported by the ATSDR (Cooperative Agreement U61/ATU881475) and NIEHS Superfund Basic Research Program (P42 ES05949).

Received 16 November 2001; accepted 20 March 2002.

TCE–GSH conjugate undergoes further biotransformation through a series of peptidases to form two isomers, *S*-1,2-dichlorovinylcysteine (*S*-1,2-DCVC) and *S*-2,2-DCVC, which are substrates for β -lyase metabolism to two reactive species, thioketene and thioaldehyde (Anders and Dekant 1998; Bruening and Bolt 2000). These latter two metabolites are believed to be responsible for the observed nephrotoxicity in rats. However, the lower level of β -lyase in human liver *in vitro* compared with rats suggests a lower contribution of this pathway in humans (Green et al. 1997). Moreover, neither the reactive metabolites themselves nor their covalent binding products have yet been identified. Despite the debate about the underlying mechanisms of renal tumor formation for TCE and PERC and their relevance for human risk assessment (Barton and Das 1996; Bogen and Gold 1997; Fisher et al. 1998), quantitative changes in TCE metabolism are expected to have a significant impact on the assessment of its overall chronic toxicity.

Over the last decade, biological monitoring has been established as a very useful tool for assessing workers' exposures to chemicals (Droz et al. 1989; Jang et al. 1997). Biomarkers of exposure such as generated metabolites and/or parent compound blood levels are measures frequently used to assess past and current exposures in occupational and environmental settings. Although not the only source of variability in the observed biological monitoring results, metabolic interactions due to mixed

exposures can significantly complicate their interpretation (Jang et al. 1999a). Coexposure to another chemical can either increase or decrease the biomarker levels (compared with single exposure) by altering metabolism and/or elimination. Knowledge about the magnitude and direction of such changes is essential for correct interpretation of the observed biological levels. This article is an enhancement of the current understanding about biological monitoring of occupational exposures to TCE and illustrates the usefulness of physiologically based pharmacokinetic (PBPK) modeling to account for metabolic interactions in assessment of biological monitoring data. Such information may be used to suggest biological limit values (BLVs) for mixed exposures.

Previous animal studies from our laboratory indicated that simultaneous exposures to TCE, PERC, and MC within their current TLVs [time-weighted average (TWA)] would result in elevated TCE blood levels compared with single chemical exposures (Dobrev et al. 2001).

To assess the significance of these findings in humans, we designed a study to enhance our understanding of the mechanistic basis of such interactions and of their thresholds and to support the process of risk assessment of chemical mixtures. Specifically, the objectives of the current work were defined as follows: *a*) development of an integrated human PBPK model to describe the individual pharmacokinetics of TCE, PERC, and MC in a ternary mixture for different occupational

exposure scenarios at and below their current TLV/TWA; *b*) evaluation of TCE blood concentration and total TCE metabolites generated as appropriate dose metrics for identifying the occurrence of pharmacokinetic interactions during combined exposures to TCE, PERC, and MC in rats and humans; *c*) calculation of interaction thresholds (i.e., external exposure concentrations resulting in a preselected level of change in internal tissue dosimetry) to provide a quantitative measure of interactions at occupationally relevant settings using model-derived estimates of TCE blood levels and total TCE metabolites as biomarkers of exposure; and *d*) quantitative assessment of the changes in parent TCE and its metabolites at the calculated thresholds, and their implications to the occupational risk assessment of exposures to chemical mixtures.

Approach and Methods

In this study, we integrated our understanding of biochemistry, physiology, and metabolic interactions with computer simulations to predict the individual kinetics of TCE, PERC, and MC in humans exposed to constant levels of differently constituted chemical mixtures of

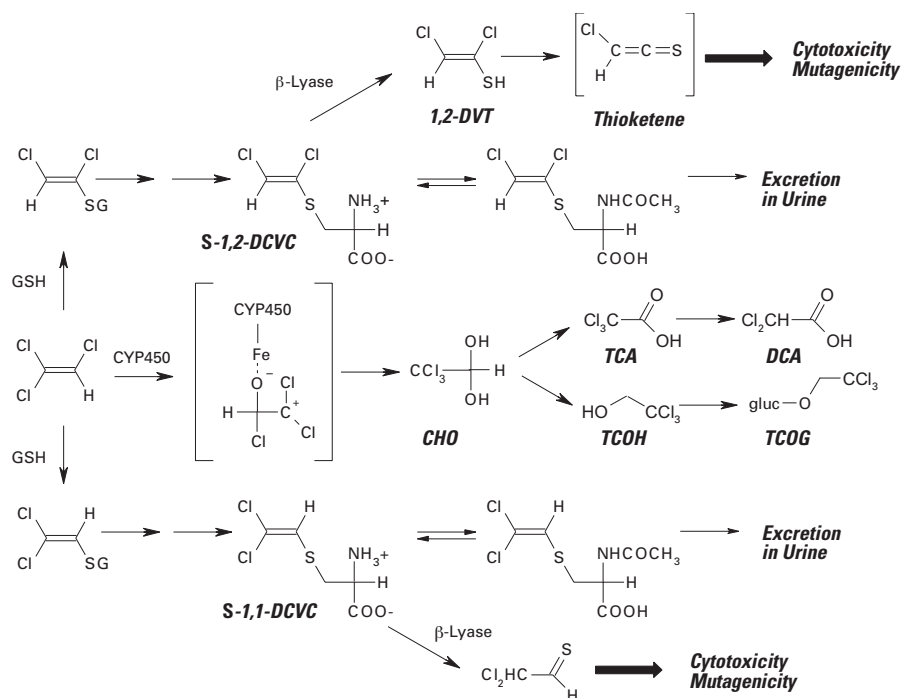


Figure 1. Metabolism of TCE. 1,2-DVT, 1,2-dichlorovinylthiol; TCOG, trichloroethanol glucuronide; TCA, trichloroacetic acid; DCA, dichloroacetic acid.

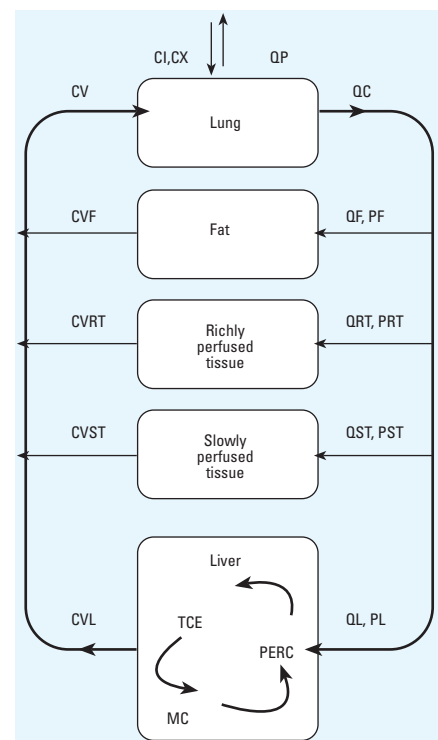


Figure 2. Ternary-mixture PBPK model representation for humans. Fat (F), richly perfused (RT), slowly perfused (ST), and liver (L) tissue groups are characterized with their volumes (Table 1), perfusion rates (QF, QRT, QST, and QL), partition coefficients (Pf, PRT, PST, and PL), and concentrations of venous blood effluents (CVF, CVRT, CVST, and CVL). CV is the mixed venous blood concentration, QC and QP are cardiac output and pulmonary ventilation, and CI and CX are inhaled and exhaled air concentrations.

the three solvents. We used the mixture model to evaluate the general pharmacokinetic profile of two common biomarkers of exposure, peak TCE blood concentrations and total amount of TCE metabolites generated, in rats and humans. We then used PBPK models for each single chemical and the integrated mixture model to calculate interaction thresholds for both species for a variety of occupationally relevant exposure scenarios. Finally, we calculated the relative changes in each biomarker of exposure at the simulated interaction thresholds in humans.

PBPK model development and parameterization. The kinetics of each solvent in the chemical mixture are described by individual PBPK models, linked via the metabolism term in the liver compartment (Andersen et al. 1987). The human body is represented as a system of five tissue compartments (liver, adipose, lung, and richly and slowly perfused tissue groups) connected through the systemic blood circulation (Figure 2). Physiologic, physicochemical, and biochemical model parameters originate from the literature and are summarized in Tables 1 and 2 (Dobrev et al. 2001; Fisher et al. 1998; Gargas et al. 1986; Reitz et al. 1988, 1996). The solvents

enter the body via inhalation, and their uptake in each tissue compartment is assumed to be blood-flow limited. Metabolism is restricted to the liver and includes oxidative cytochrome P450 (CYP450)-mediated pathway (a saturable Michaelis-Menten process) and a first-order elimination process describing the GSH conjugation of TCE (Figure 1). Competitive inhibition is incorporated into the saturable metabolism term of each chemical to account for the coexposure effects of the other two mixture components as described for the rat (Dobrev et al. 2001). However, because of limitations in detecting possible changes in PERC and MC metabolism caused by TCE coexposure, only inhibition of TCE metabolism was implemented through the use of appropriate inhibition constants (i.e., $K_i = 100,000$ for PERC and MC). Parameterization of the GST-mediated pathway was from the work of Clewell et al. (2000) and includes a first-order process for production of both S-1,2- and S-2,2-DCVC isomers in the liver. The rate constant for DCVC generation (K ; Table 2) in the Clewell et al. model is estimated from measurements of oxidative and conjugative metabolites in urine of human volunteers after TCE exposures (Bernauer et

al. 1996). In its present form, the mixture model accounts for changes in DCVC production rates of potentially cytotoxic/genotoxic metabolites; however, further activation and/or detoxification processes are not included (Clewell et al. 2000). Because rates for GSH conjugation of TCE in humans are reported to be 1,000–7,000 times lower than the oxidative rates of metabolism (Bloemen et al. 2001), the calculation of total metabolites generated as an indication for occurrence of metabolic interactions was based only on the saturable oxidative pathway (see “Results and Discussion”).

Allometric scaling was applied to rates of metabolism (V_{max} , K), cardiac output (QC), and pulmonary ventilation (QP) to account for differences in body weight (Krishnan et al. 1994a). Apparent affinity constants (K_m) and inhibition constants (K_i) are considered enzyme-specific parameters and used with no modification. Model differential equations (provided in Appendix) are solved using ACSL (Advanced Continuous Simulation Language) version 11.5 (AEGIS Simulation, Inc., Huntsville, AL) and Berkeley Madonna (Berkeley, CA) simulation software packages.

Individual model calibration. Our human mixture model integrates previously developed, validated, and extensively studied individual PBPK models for TCE, PERC, and MC. The performance and potential limitations of these single chemical models are discussed elsewhere (Fisher 2000; Reitz et al. 1988, 1996). Because parameterization of our mixture model originates from formerly validated human models, no experimental verification of either the model parameters or the single-model simulations described in the literature source was undertaken in this study. Instead, we used available literature data on human exposures to each single chemical to evaluate the overall performance of the individual PBPK models (Fernandez et al. 1976; Fisher et al. 1998; Monster et al. 1979; Reitz et al. 1988). From a variety of published data, we selected parent compound blood and expired air concentrations as direct and sensitive descriptors of the chemicals' pharmacokinetics. Good agreement between single-model simulations and kinetic data from four different laboratories covering the period 1976–1998 was achieved without further statistical optimization (Figures 3–5). We performed model calculations for the exposure conditions specified in each particular work and a standard body weight of 70 kg.

Interaction threshold calculations. Inhibitors will cause some changes in the substrate's kinetics at any exposure level. However, inhibition at very low concentrations will not be quantitatively significant. We defined the interaction threshold as the minimal level of change in the tissue dosimetry

Table 1. Physiologic parameters for rats and humans used in the ternary-mixture PBPK model.

Physiologic parameters	Human	Rat
Alveolar ventilation (L/hr/kg)	15.0	15.0
Cardiac output (L/hr/kg)	15.0	15.0
Blood flow as a fraction of total cardiac output		
Blood flow to liver	0.25	0.25
Blood flow to richly perfused tissue	0.449	— ^a
Blood flow to slowly perfused tissue	0.249	— ^a
Blood flow to fat	0.052	0.09
Blood flow to lumped tissue	— ^a	0.66
Compartment volumes as fraction of body weight		
Fraction slowly perfused	0.651	— ^a
Fraction richly perfused	0.118	— ^a
Fraction fat	0.214	0.09
Fraction liver	0.026	0.04
Fraction lumped tissue	— ^a	0.78

^aRat PBPK model uses a three-compartment structure (Dobrev et al. 2001).

Table 2. Partition coefficients and biochemical constants for rats and humans used in the ternary-mixture PBPK model.

	TCE		PERC		MC	
	Human ^a	Rat ^b	Human ^c	Rat ^b	Human ^d	Rat ^d
Partition coefficients						
Blood/air	11.2	22.0	10.3	18.9	2.5	5.8
Fat/blood	52.3	25.3	119.1	86.9	104.0	45.7
Liver/blood	4.9	1.2	5.9	3.7	3.4	1.5
Richly perfused tissue/blood	1.1	— ^e	5.9	— ^e	3.4	— ^e
Slowly perfused tissue/blood	1.4	— ^e	3.1	— ^e	0.6	— ^e
Lumped tissue/air	— ^e	0.5	— ^e	1.1	— ^e	0.6
Biochemical constants						
V_{max} (mg/hr/kg)	4.0	11.0	1.42	0.93	0.42	0.42
K_m (mg/L)	1.80	0.25	4.66	5.62	5.75	5.75
K_i (mg/L) ^f	— ^g	— ^g	6.3	6.3	4.2	4.2
K (hr)	0.015 ^h					

^aFisher et al. 1998. ^bGargas et al. (1986). ^cCalculated from Reitz et al. (1996). ^dReitz et al., 1988. ^eRat PBPK model uses three-compartment structure [see Dobrev et al. (2001)]. ^fDobrev et al. (2001; rat estimates). ^gInhibition effect of TCE on PERC and MC metabolism (Dobrev et al. 2001). ^hClewell et al. 2000.

associated with potentially adverse health effects (Dobrev et al. 2001; El-Masri et al. 1996a, 1996b). A 10% or more increase in TCE blood concentration or a 10% or more decrease of the total TCE metabolites generated as a direct result of mixture coexposure was proposed as a criterion for the occurrence of chemical interaction (Dobrev et al. 2001). The selected level of alteration (10%) illustrates a potential application of our modeling approach and can be easily changed for any particular purpose. We performed model simulations using two mechanistic models (competitive inhibition and no interaction) for constant exposures of rats and humans to different solvent levels and combinations. Comparing the output of the two mechanistic models at the end of the 6-hr exposure period allowed calculation of the interaction thresholds.

Results

Using the integrated human mixture model, we investigated the pharmacokinetic profiles of two common biomarkers of exposure, peak TCE blood levels and total amount of TCE metabolites generated, over a broad range of PERC and MC coexposure concentrations. In addition, we used changes in the production rates of metabolites formed by GSH conjugation to quantitatively assess the impact of increased TCE blood concentrations on the potential metabolic shift toward this generally minor biotransformation pathway.

In each simulated experiment, calculations were performed for a constant 6-hr occupational TLV exposure to TCE (50 ppm) and gradually increasing PERC and MC concentrations (up to 2,000 ppm). The results at the end of the 6-hr exposure period are summarized as dose-response surface plots in Figures 6–8 and illustrate the general pharmacokinetic behavior of these dose measures within the investigated concentration range. The simulated peak TCE blood level at the end of a single chemical exposure to 50 ppm was 1.29 mg/L; this value would increase to 1.83 mg/L (a 42% change) during the coexposure concentrations of PERC and MC up to 2,000 ppm (Figure 6). The maximal change in this biomarker (i.e., assuming complete inhibition of TCE metabolism) was calculated at 1.92 mg/L (49% increase), and this value was not reached in simulated exposures up to 20,000 ppm of PERC and MC (1.91 mg/L; data not shown).

The change in the total TCE metabolites generated followed a similar pattern, although in the opposite direction: suppression of TCE metabolism due to mixture coexposure logically resulted in decreased metabolite formation (Figure 7). Starting at 166.4 mg total metabolites [2.4 mg/kg body weight (BW)] for exposure to TCE alone, this biomarker

would decrease to 26.8 mg (0.38 mg/kg BW) at PERC and MC coexposure concentrations of 2,000 ppm. This fractional change of 84% (vs. single TCE exposure) is considerably higher than the observed increase in parent compound blood concentration, indicating a better sensitivity of metabolite generation to detect chemical interactions. As demonstrated with the peak blood levels, simulations at very high coexposure levels of PERC and MC (up to 20,000 ppm) did not completely inhibit TCE metabolism (data not shown).

Model output for the expected changes in the amount of DCVC metabolites is summarized in Figure 8. For a single chemical exposure to 50 ppm TCE, the model predicted generation of 64 μ g (0.9 μ g/kg BW) DCVC derivatives at the end of the 6-hr exposure period. This amount is approximately 3,000 times less than the simulated total oxidative TCE metabolites for the same exposure conditions (166 mg; Figure 7) and is in general agreement with the reported quantitative differences in both metabolic pathways (Bloemen

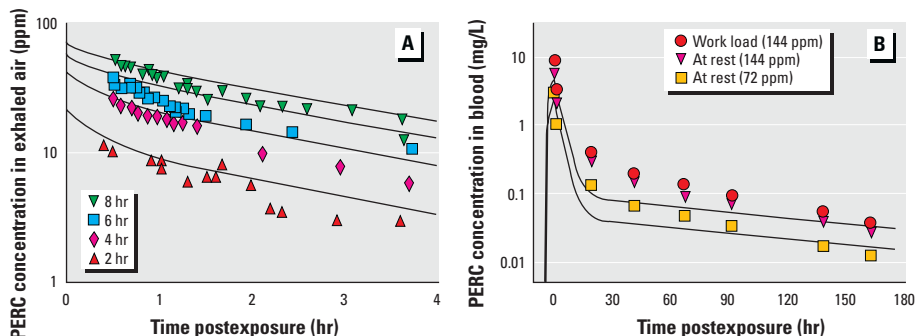


Figure 3. Evaluation of the single PERC PBPK model performance: model simulations versus literature data. (A) PERC concentration in exhaled air, after exposure to 200 ppm for 8, 6, 4, and 2 hr (Fernandez et al. 1976). (B) Venous blood concentration during and after 4-hr exposures to 72 and 144 ppm PERC with and without workload (Monster et al. 1979). Symbols are measured data, and lines are model simulations.

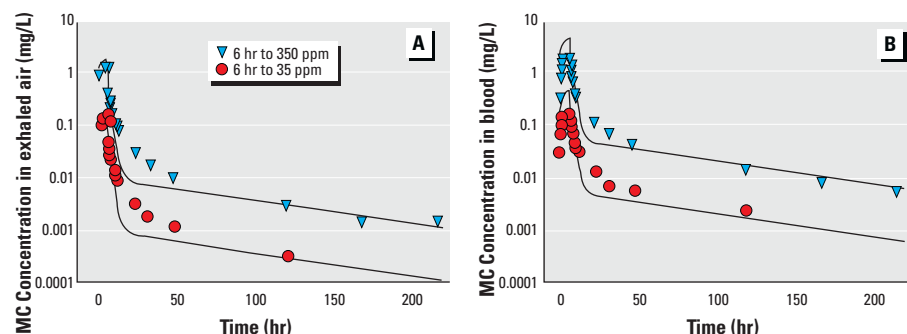


Figure 4. Evaluation of the single 1,1,1-trichloroethane PBPK model performance: model simulations versus literature data (Reitz et al. 1988). MC concentration in exhaled air (A) and venous blood (B) during and after 6-hr exposure to 35 and 350 ppm MC. Symbols are measured data, and lines are model simulations.

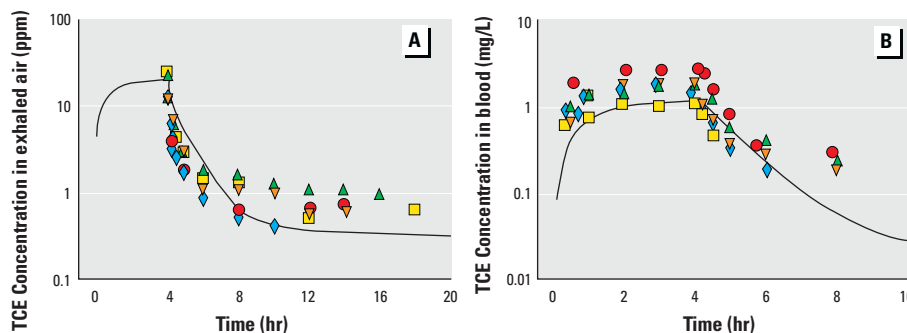


Figure 5. Evaluation of the single TCE PBPK model performance: model simulations versus literature data (Fisher et al. 1998). TCE concentration in exhaled air [(A) 4-hr exposure to 100 ppm] and venous blood [(B) 4-hr exposure to 50 ppm]. Different symbols are measured data in different subjects, and lines are model simulations.

et al. 2001). In contrast to the pharmacokinetic profile of the oxidative metabolites (Figure 7), inhibition of TCE metabolism in the presence of PERC and MC significantly increased the total DCVC products (Figure 8). Such an increase is directly related to the elevated blood levels of the parent chemical (Figure 6), and for coexposure concentrations of 1,000 ppm PERC and MC, the amount of DCVC metabolites increased more than 64%, reaching 105 μg (1.5 $\mu\text{g}/\text{kg BW}$). Under the hypothetical conditions of complete metabolic inhibition (i.e., lack of oxidative TCE metabolism), the upper limit for DCVC metabolite generation was calculated at 121 μg (1.7 $\mu\text{g}/\text{kg BW}$).

With respect to the practical application of our work to biological monitoring of occupational exposures, we calculated interaction thresholds in rats and humans using TCE blood levels and total TCE metabolites as biomarkers of combined exposures to the three solvents. Modeling results for selected occupationally relevant exposures to TCE in combination with PERC and/or MC (implementing no-interaction and competitive-inhibition mechanistic models) and the calculated interaction thresholds in humans are summarized in Table 3. Using our previously reported mixture model (Dobrev et al. 2001), interaction thresholds in rats were calculated for comparable exposure scenarios (Table 4). Simulated

TLV exposures to the three solvents (i.e., 25/50/350 ppm of PERC/TCE/MC) predicted a 15% (human) and 22% (rat) increase in the peak TCE blood concentration, and a 29% (human) and 6% (rat) decrease in the total TCE metabolites generated compared with single chemical exposures. Binary TLV exposures to TCE/PERC mixtures did not significantly affect any biomarker in either species, whereas interaction thresholds for human TCE/MC exposures were reached at 50 ppm TCE and 230 ppm MC (TCE blood levels), and 50 ppm TCE and 100 ppm MC (total TCE metabolites), respectively.

Keeping TCE and PERC concentrations at their current TLV/TWA levels, interaction thresholds in humans for the ternary mixture of TCE/MC/PERC were calculated at 50/180/25 ppm (TCE blood levels) and 50/65/25 ppm (total TCE metabolites), respectively (Table 3). Under the same exposure conditions, interaction thresholds in rats were calculated at 50/130/25 ppm TCE/MC/PERC using TCE blood levels as a biomarker of exposure (Table 4). The change in total TCE metabolites predicted by the rat model did not exceed the 10% significance level within the selected TLV evaluation range.

Discussion

Our laboratory has been practicing “*in silico*” experimentation for about 10 years. In essence, it means integrating computer modeling with focused, mechanistic, animal experimentation such that experiments that are impractical (e.g., too large, too expensive) or impossible (e.g., human experiments with carcinogens) to perform are conducted on computer. Earlier examples of *in silico* experimentation from our laboratory, using PBPK/pharmacodynamic modeling and other computer modeling techniques such as Monte Carlo simulation, include *a*) acute toxicity studies, at 1,000 rats per group, of toxicologic interactions between Kepone and carbon tetrachloride (El-Masri et al. 1996c); *b*) human biological exposure index studies on six industrial solvents (Thomas et al. 1996a); *c*) interaction threshold studies on binary and ternary chemical mixtures (Dobrev et al. 2001; El-Masri et al. 1996a, 1996b); *d*) studies on age- and dosing-related pharmacokinetic changes in mice in a 2-year chronic toxicity/carcinogenicity bioassay (Thomas et al. 1996b); and *e*) clonal growth modeling of early stages of carcinogenesis (Ou et al. 2001, 2002). We believe that use of computer simulation is essential in the studies of chemical mixture toxicology and risk assessment. The area of toxicology, in general, will be well served by the application of computer technology as an alternative research method to minimize the killing of laboratory animals.

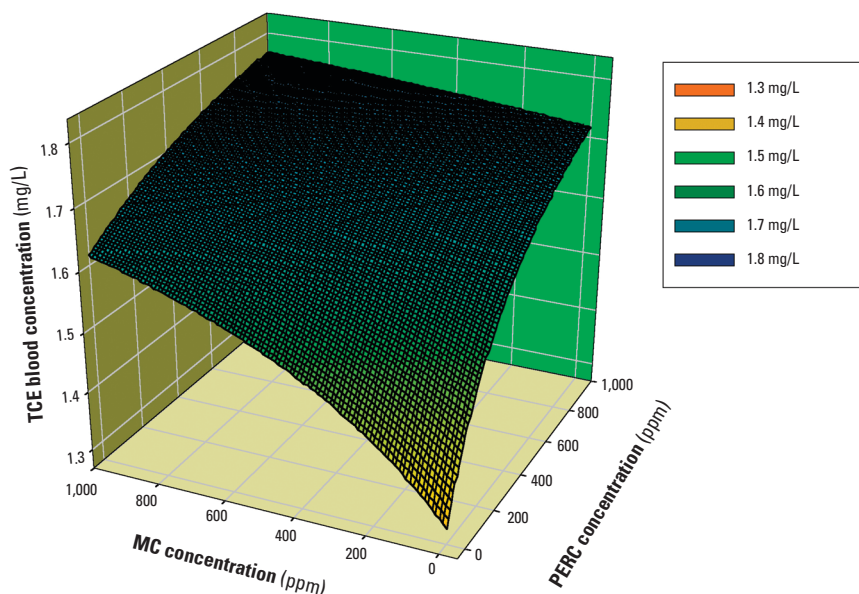


Figure 6. Pharmacokinetic profile of peak TCE blood concentration in humans at the end of a constant 6-hr TLV exposure (50 ppm) to TCE for various PERC and MC concentrations.

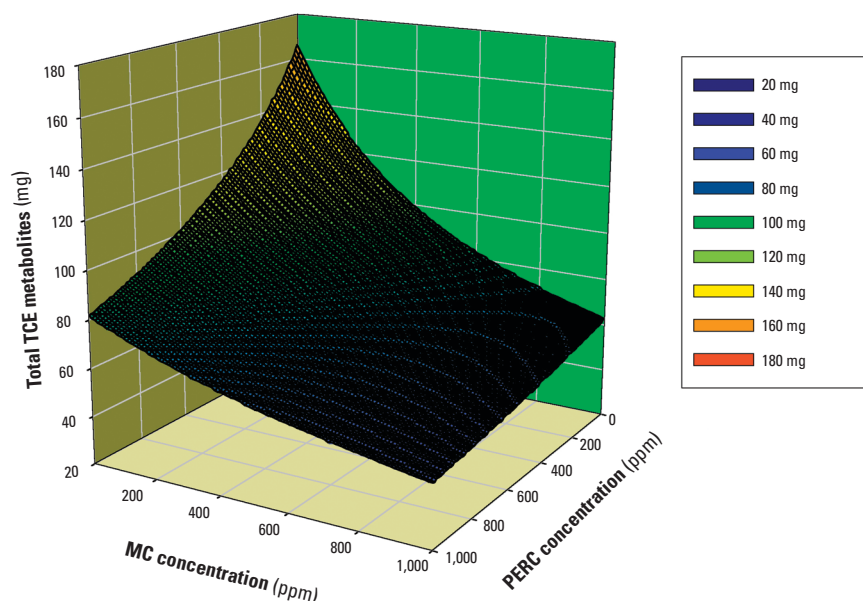


Figure 7. Pharmacokinetic profile of total TCE metabolites generated in humans at the end of a constant 6-hr TLV exposure (50 ppm) to TCE for various PERC and MC concentrations.

Combined exposures to multiple chemicals have been shown to significantly affect the pharmacokinetics of one or more mixture components and alter the target tissue dose (Andersen et al. 1987; El-Masri et al. 1996a, 1996b; Pelekis and Krishnan 1997; Tardif and Charest-Tardif 1999; Tardif et al. 1993, 1995). A previously reported rat study from our laboratory indicated that occupational exposures to chemical mixtures of the three solvents within their TLV/TWA limits would result in a significant increase (22%) in the TCE blood levels compared with single chemical exposures (Dobrev et al. 2001). In the present study, we have developed and implemented an integrated human PBPK model to assess the significance of these findings for human occupational exposures to TCE, PERC, and MC. Our calculations show that

simulated TLV exposures to TCE, PERC, and MC (50, 25, and 350 ppm, respectively) are expected to produce a 15% increase of the TCE blood concentration compared with single chemical exposure (Table 3; 1.48 vs. 1.29 mg/L). The health significance of such elevation in TCE dose might become even more important with respect to the presence of the other two solvents in the chemical mixture, which have similar sedative effects on the central nervous system (and are expected to be additive at low doses). Currently, exposures to mixtures of organic solvents are usually calculated as cumulative exposure indices (CEIs) by adding the ratios of biological monitoring results to the respective BLVs (Jang et al. 1999b, 2001). As shown here, CEIs developed without considering possible pharmacokinetic interactions may significantly

underestimate exposure. Therefore, it is especially important to review the BLVs of organic solvents concerning their metabolic interactions (Jang et al. 2001). If exposure to one chemical is unlikely, and coexposure is common in most occupational exposures, metabolic interactions should be considered in applying and developing BLVs.

Long-term health effects of TCE, on the other hand, are clearly associated with its biotransformation products (Barton and Clewell 2000; Bull 2000; Lash et al. 2000). Hence, a detailed understanding and quantitative assessment of the changes in TCE metabolism in chemical mixtures become particularly important for the risk assessment of chronic exposures to this solvent. Of oxidative and conjugative biotransformation products (Figure 1), genotoxic and nephrotoxic DCVC isomers appear to be the most relevant for the recorded chronic toxicity of TCE. In a recently reported study, an increased incidence of renal tumors was observed in a cohort of workers occupationally exposed to high TCE concentrations for more than 20 years (Henschler et al. 1995). The slow excretion rate of these metabolites is likely to result in a more important contribution of this generally minor pathway in TCE and PERC metabolism, especially with long-term occupational exposures. In addition, some nonlinearities in the conjugative biotransformation of PERC observed in rats, nonevident in TCE metabolism (Bernauer et al. 1996), may become more prominent at conditions inhibiting the CYP450 oxidative pathway. Therefore, pharmacokinetic factors that increase the flux through this metabolic pathway could substantially contribute to significantly increased risk of kidney tumor formation in exposed populations. Recent work on a five component chemical mixture predicted up to 4-fold increases in cancer risk for combined exposures to dichloromethane due to increased metabolic flux through the GSH conjugation pathway (Haddad et al. 2001).

Our results further exemplify the importance of such analysis for risk assessment of chemical mixtures. Elevated TCE blood levels, as a result of multiple exposures, will consequently lead to higher TCE bioavailability for GSH conjugation. In general, the pharmacokinetic profile of DCVC metabolites follows the pattern demonstrated for the change in the TCE blood levels for mixed exposures (Figures 6 and 8). Such pharmacokinetic behavior can be logically expected, because the model structure links linearly the production rates of DCVC to the liver concentration of TCE (Clewell et al. 2000). However, TCE blood levels in the liver are nonlinearly correlated to saturable Michaelis-Menten kinetics of the quantitatively predominant oxidative TCE metabolism. Thus,

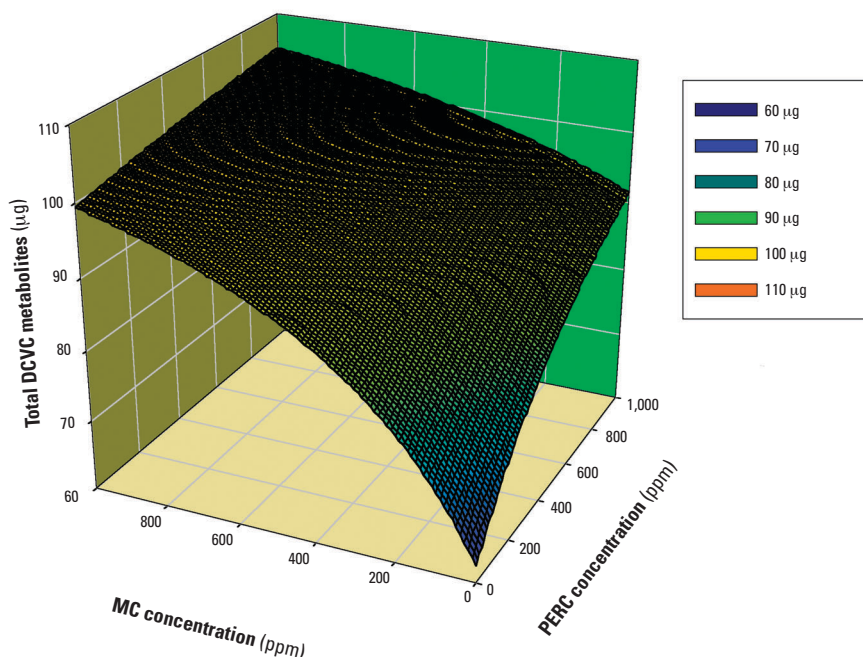


Figure 8. Pharmacokinetic profile of total DCVC metabolites generated in humans at the end of a constant 6-hr TLV exposure (50 ppm) to TCE for various PERC and MC concentrations.

Table 3. Interaction thresholds in humans for occupationally relevant exposures to TCE, MC, and PERC.

TCE	Exposure level (ppm)		TCE blood conc (mg/L)		Total TCE metabolites (mg)	
	MC	PERC	Comp inhibition	No interaction	Comp inhibition	No interaction
50	350	0	1.46	1.29	120.84	166.42
50	300	0	1.44	1.29	130.86	166.42
50*	230*	0*	1.42*	1.29*	125.64	166.42
50	130	0	1.37	1.29	145.59	166.42
50*	100*	0*	1.35	1.29	149.87*	166.42*
50	350	25	1.48	1.29	117.49	166.42
50	250	25	1.44	1.29	126.88	166.42
50*	180*	25*	1.42*	1.29*	135.06	166.42
50	130	25	1.39	1.29	140.55	166.42
50	100	25	1.37	1.29	144.48	166.42
50*	65*	25*	1.36*	1.29	149.40*	166.42*
50	0	25	1.32	1.29	159.58	166.42

Abbreviations: comp, competitive; conc, concentration. The simulated exposure and biomarker levels at which a significant metabolic interaction is likely to occur are highlighted. "No interaction" values are calculated for single TCE exposures using the mixture model (MC and PERC concentrations are zero).

*Interaction thresholds.

a fractional change in the TCE blood concentration of 15% for combined TLV exposure to the three chemicals (25/50/350 ppm of PERC/TCE/MC) results in a 27% increase of the DCVC metabolites (Table 5). Similarly, binary combinations of the solvents produced GST-mediated metabolite levels almost twice as high as the expected rates of increase in the parent compound blood levels (Table 5). Further comparison with the simulated interaction thresholds in humans (Table 5) indicates 17–18% increased DCVC products at the 10% significance level using TCE blood concentration as a biomarker of exposure. In the same time, the simulated increase of DCVC metabolites remained 9% for interaction thresholds derived from total TCE metabolites as a biomarker of exposure (Table 5). Such differences illustrate the importance of selecting appropriate biomarkers of exposure in the risk assessment of chemical mixtures.

Biomarkers of exposure, such as reduced metabolite generation and/or increased blood concentration of the parent compound, are widely used dose measures for identifying pharmacokinetic interactions during and after exposure to chemical mixtures. Recent work demonstrated the differential sensitivity

of each biomarker toward detection of metabolic interactions and its application to research involving chemical mixtures (Tardif and Charest-Tardif 1999). Experimental data and pharmacokinetic analysis at steady state illustrated that combined exposures to MC and *m*-xylene did not affect MC blood levels but significantly reduced the formation and excretion of two MC metabolites, TCOH and trichloroacetic acid (Tardif and Charest-Tardif 1999). Therefore, one of the objectives of our study was to evaluate these two biomarkers of exposure, namely, TCE blood levels and total TCE metabolite formation, as appropriate measures for occurrence of pharmacokinetic interactions in occupational settings.

For the selected mixture, both dose metrics can be used as biomarkers of chemical interaction. At a 10% significance level, earlier detection of interaction thresholds (i.e., at lower exposure levels) would be possible based on decreased TCE metabolite formation as a biomarker of exposure rather than using parent compound levels (Table 3). Our approach illustrates the importance of investigating and selecting the appropriate dose measure in the overall process of risk assessment for these mixtures. Particularly, the difference in the

estimated 10% interaction threshold for MC coexposure is 65 versus 180 ppm in ternary and 100 versus 230 ppm in binary mixtures, depending on the biomarker used (Table 3). Choosing the less sensitive biomarker (parent blood levels in this case) would result in two to three times higher estimates of potentially safe exposure levels. In addition, assuming DCVC metabolite generation as the main adverse health effect for long-term TCE exposures, interaction thresholds based on total TCE metabolites will be more appropriate, because quantitatively they correspond more closely with the expected changes in DCVC production rates (Table 5). For instance, interaction thresholds (i.e., solvent concentrations in air) calculated using a 10% increase in parent TCE blood levels would correspond to an 18% rise in DCVC formation (roughly twice the preselected threshold level of 10%), whereas interaction thresholds based on a 10% decrease in total TCE metabolites correspond to a 9% increase in DCVC metabolites.

In the course of this study, important species-specific differences became evident. In rats, TCE blood concentration is the more sensitive parameter to detect the metabolic inhibition effects of PERC and MC (Figure 9), whereas in humans the total of TCE

Table 4. Interaction thresholds in rats for occupationally relevant exposures to TCE, MC, and PERC.

Exposure level (ppm)			TCE blood conc (mg/L)		Total TCE metabolites (mg)	
TCE	MC	PERC	Comp inhibition	No. interaction	Comp inhibition	No interaction
50	350	0	1.20	1.00	6.57	6.93
50	300	0	1.17	1.00	6.61	6.93
50	200	0	1.12	1.00	6.72	6.93
50*	170*	0*	1.10*	1.00*	6.75	6.93
50	100	0	1.06	1.00	6.82	6.93
50	350	25	1.22	1.00	6.53	6.93
50	250	25	1.17	1.00	6.63	6.93
50	180	25	1.13	1.00	6.70	6.93
50*	130*	25*	1.10*	1.00*	6.75	6.93
50	65	25	1.06	1.00	6.82	6.93
50	0	25	1.02	1.00	6.89	6.93

Abbreviations: comp, competitive; conc, concentration. The simulated exposure and biomarker levels at which a significant metabolic interaction is likely to occur are highlighted. "No. interaction" values are calculated for single TCE exposures using the mixture model (MC and PARC concentrations are zero). A 10% interaction threshold based on total TCE metabolites generated would be reached at 6.24 mg.

*Interaction thresholds.

Table 5. Increased generation of toxic GSH metabolites (compared with single-chemical exposure) for combinations of binary and ternary TLV exposures to TCE, PERC, and MC, and their relation to the calculated interaction thresholds in humans.

Exposure level (ppm)			TCE blood conc (mg/L)	CYP450 metabolites (mg)	GSH metabolites (μg)
TCE	MC	PERC			
50	0	0	1.29	166.42	62.4
50	350	25	1.48 (15%)	117.49 (29%)	79.0 (27%)
50	350	0	1.46 (13%)	120.84 (27%)	77.7 (24%)
50	0	25	1.32 (2%)	159.58 (4%)	64.8 (4%)
50*	230*	0*	1.42*	125.64	73.5 (18%)
50*	100*	0*	1.35	149.87*	67.9 (9%)
50*	180*	25*	1.42*	135.06	73.2 (17%)
50*	65*	25*	1.36	149.40*	68.2 (9%)

conc, concentration. The simulated exposures at which a significant metabolic interaction is likely to occur and the corresponding biomarker levels are highlighted. Values in parenthesis indicate a fractional change in the biomarker levels and GSH metabolites. See "Discussion" for more details.

*Interaction thresholds.

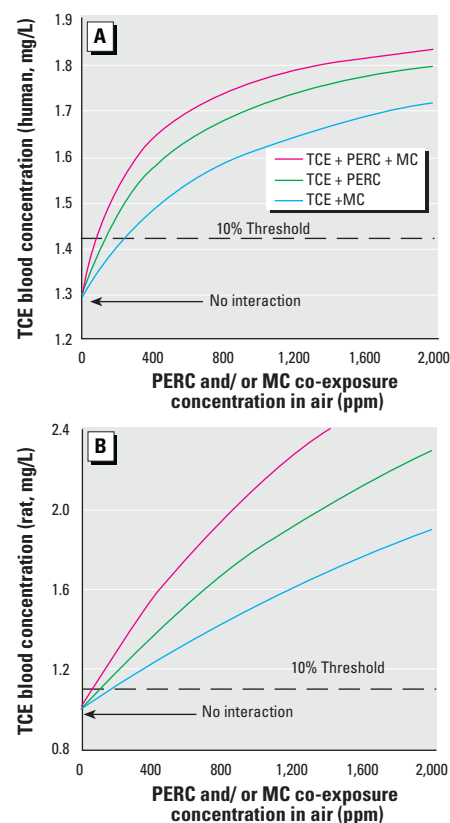


Figure 9. Evaluation of TCE blood concentration at the end of a constant 6-hr TLV exposure (50 ppm) to TCE as a biomarker for the occurrence of chemical interactions in binary and ternary mixture combinations of TCE, PERC, and MC in (A) human and (B) rat.

metabolites produced is a more sensitive dose measure (Figure 10). The bases for such discrepancies are species differences in physiology and/or the chemical-specific properties of TCE. The blood/air partition coefficient of TCE in rats is two times higher than in humans (22 vs. 11; Table 2), and the rate of metabolism is three times higher in rats than in humans (11 vs. 4 mg/hr/kg; Table 2). In addition, physiologic differences in perfusion and ventilation rates, as well as the size of fat and liver compartments, may play an important role in the observed phenomenon. These factors were readily evaluated by *in silico* simulation with the PBPK model.

Generally, interaction thresholds in humans exposed to ternary mixtures of TCE, PERC, and MC are expected to occur at lower exposure concentrations than in rats (Figures 9 and 10). Toxicologically, the consequences of the observed shifts toward lower metabolite and higher parent compound concentrations in blood remain to be investigated. Elevated TCE blood concentrations are clearly associated with suppression of the central nervous system. During combined exposures, such effects are expected to occur at lower exposure levels compared with single TCE exposure. In addition, changes in the metabolism pattern

will lead to higher bioavailability of TCE for the GSH metabolic pathway, resulting in higher risk of kidney damage.

Our results exemplify the *a priori* use of PBPK modeling for designing interaction studies and identifying sensitive end points.

Appendix

A series of mass balance differential equations were used to build the ternary mixture PBPK model. For each compartment, an equation describes the uptake, metabolism (in liver only), and elimination for each of the three mixture components. Uptake in each nonmetabolizing tissue group (fat, slowly and richly perfused compartments) is a blood-flow-limited process governed by the perfusion rate of the particular compartment (Q_T , as L/hr), the tissue/blood partition coefficient (P_T), and the arterial concentration (C_A , as mg/L):

$$V_T \times \frac{dC_T}{dt} = Q_T \times \left(C_A - \frac{C_T}{P_T} \right). \quad [1]$$

Here, C_T is each chemical's concentration (mg/L) in a nonmetabolizing tissue compartment with volume V_T (L).

Metabolism occurs in the liver using an additional term to account for the rate of change in the metabolized amount,

$$\frac{dAM}{dt},$$

added to Equation 1:

$$V_L \times \frac{dC_L}{dt} = Q_L \times \left(C_A - \frac{C_L}{P_L} \right) - \frac{dAM}{dt}. \quad [2]$$

Metabolism of TCE, PERC, and MC in the liver follows saturable Michaelis-Menten kinetics. Assuming competitive inhibition as the predominant mechanism of pharmacokinetic interaction related to combined exposures (Andersen et al. 1987), the rate of saturable metabolism

$$\frac{dAM}{dt}$$

for each solvent is given by

$$\frac{dAM_i}{dt} = \frac{V_{maxi} \times C_{Li} / P_{Li}}{K_{mi} \times \left(1 + \frac{C_{L2} / P_{L2}}{K_{i2}} + \frac{C_{L3} / P_{L3}}{K_{i3}} \right) + C_{Li} / P_{Li}}. \quad [3]$$

Further, interactive model exercises will allow estimation of safe exposure levels during multiple chemical exposures. The approach illustrates a novel application of PBPK modeling to predict the occurrence of metabolic interactions during mixed exposures and quantitatively

Subscripts 1, 2, and 3 indicate parameters related to compounds 1, 2, and 3 in the chemical mixture. V_{max} (mg/hr/kg) and K_m (mg/L) are the maximum rate of metabolism and the apparent affinity constant, respectively; K_i is the inhibition constant (mg/L), and P_L is the liver/blood partition coefficient for each component.

For TCE, the production rate of GST-mediated DCVC metabolites,

$$\frac{dAM_{GST}}{dt}$$

is calculated as the product of a first-order rate constant K (/hr), the liver volume V_L (L), and the TCE concentration in the liver (C_L , mg/L):

$$\frac{dAM_{GST}}{dt} = V_L \times C_{L1} \times K. \quad [4]$$

Accordingly, the total mass balance of TCE in the liver is

$$V_L \times \frac{dC_L}{dt} = Q_L \times \left(C_A - \frac{C_L}{P_L} \right) - \frac{dAM}{dt} - \frac{dAM_{GST}}{dt}. \quad [5]$$

The concentration C_A (mg/L) of each solvent in the arterial blood leaving the lung compartment depends on pulmonary ventilation (Q_P) and cardiac output (Q_C) rates, the venous blood concentration (C_V) entering the lungs, the blood/air partition coefficient (P_B), and the atmospheric concentration C_{air} (mg/L):

$$C_A = \frac{(Q_C \times C_V + Q_P \times C_{air})}{\left(Q_C + \frac{Q_P}{P_B} \right)}. \quad [6]$$

The venous blood effluents $Q \times C_V$ of all tissue compartments combine to yield the flow-averaged mixed venous concentration, C_V , of each solvent:

$$C_V = \frac{(Q_L \times C_{VL} + Q_R \times C_{VR} + Q_S \times C_{VS} + Q_F \times C_{VF})}{Q_C}. \quad [7]$$

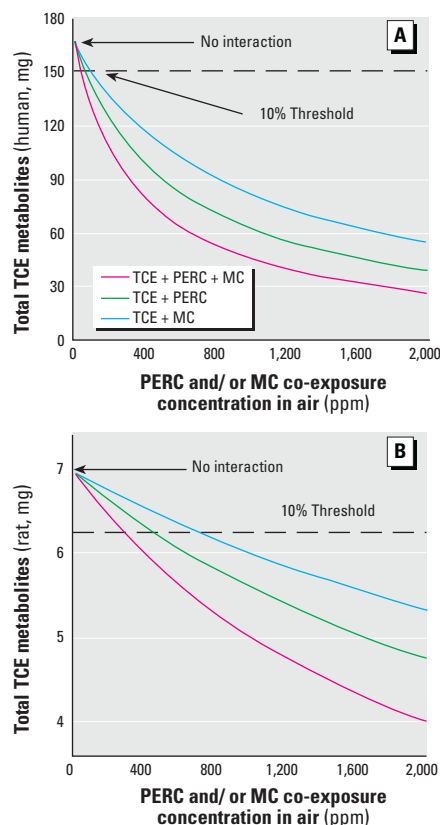


Figure 10. Evaluation of total TCE metabolites generated at the end of a constant 6-hr TLV exposure to TCE as a biomarker for the occurrence of chemical interactions in ternary mixtures of TCE, PERC, and MC in (A) human and (B) rat.

assess their impact on the kinetics of each individual mixture component. The nonlinear relationships of total GSH metabolites and TCE blood concentration and human/animal differences in response to these mixtures are important predictions that should be evaluated by appropriate experimentation.

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